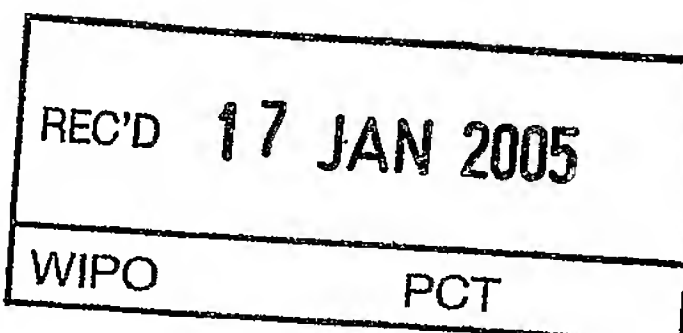


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SÖKANDE:

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UPPFINNINGENS BENÄMNING: SURFACE PROTECTION OF EXPOSED  
BIOLOGICAL TISSUES

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The present invention refers to surface protection of exposed surfaces of biological tissues. More specifically, the invention refers to a cationic polymer and an anionic polymer in combination for use in human and/or veterinary medicine for surface protection of exposed surfaces of biological tissues, especially for preventing fibrinous and fibrous adhesions of tissues.

Tissue surfaces need protection depending on the type and extent of wearing that they normally are subjected to. Most tissues within the body are thus covered by a cell membrane bilayer that mainly consists of lipids and proteins. However, epithelial surface tissues of respiratory, gastrointestinal, and to some extent the genitourinary tracts, are normally exposed to a rather harsh environment. These epithelial surfaces are further covered by a mucus layer having viscoelastic and pronounced protecting properties. Such tissues as synovia and mesothelium, on the other hand, are not protected by mucus since they are not exposed to drastic conditions of the same magnitude.

In addition, the vital epithelial tissues, such as blood vessels or blood organs, are coated with mucous, serous, synovial and endothelial membranes so that they can function independently of each other. The peritoneal, pericardial and pleural membranes consist of a single layer of mesothelial cells, which is covered with a thin film of peritoneal fluid. The components of the membranes as well as the covering layer of fluid have several functions, e.g. lubrication of the enclosed organs, unrestricted mobility being provided.

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The protective epithelial membrane is very thin and comprises a delicate layer of connective tissue covered with a monolayer of mesothelial cells and only one or a few bilayers of mainly phospholipids. This makes such tissues as synovia and mesothelium especially vulnerable to infection and trauma. When such a membrane is exposed to a physical, chemical or microbial challenge, many potent substances are often released in response thereto, which are harmful to the membrane. The structure and function of the membrane is consequently easily destroyed in connection with trauma, ischemia, and infection. After an irritation of the stress-sensitive membrane, e.g. only by the desiccation or abrasion of the membrane surfaces during surgery, it will rapidly be covered with a fibrin clot. Since the plasminogen activating activity (i.e. the fibrinolytic capacity) is reduced after trauma, the fibrin clots will later on become organized as fibrous adhesions, i.e. small bands or structures, by which adjacent serous or synovial membranes adhere in an abnormal way. Surgical operations, infection or inflammation in those parts of the body, which are coated with serous or synovial membranes, can result in adhesive inflammation regardless of the size of the affected area. The adhesions between vital epithelial tissues are formed within the first few days following surgery trauma or infection and may be observed not only in particular portions of the body but in all vital tissues. Such adhesions between for example contact zones intestines or intestines and the abdominal wall are the result of the often unnoticed tissue damage as desiccation and they occur for various reasons, including mechanical and chemical stimulations of vital tissues accompanying surgical manipulations, postoperative bacterial infection, inflammation or further complications.

Adhesion of vital epithelial tissues, large or small, may be observed in most surgical fields. It has been repor-

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ted that of all patients undergoing abdominal surgery at one hospital over a four-year period, 93 % were found to have adhesions from previous operations. In addition, in a 10 year period there will be a need of adhesion prevention in about 20% of all surgical operations, which corresponds to more than 1 million operations annually on each major continent.

However, the postsurgical adhesions obtained are the result of a natural wound healing response of tissue damage occurring during surgery. Numerous factors play a role in peritoneal wound healing and the development of adhesions. Among others are peritoneal macrophages known to have an important role in initial peritoneal repair.

Thus, while waiting after surgery for the body to produce new protective layers it is important to supply the corresponding protection from the outside to exposed epithelial surfaces in an effective way. Furthermore, it is important to prevent or reduce the infection and/or the inflammation obtained after surgery as well as the accompanying fibrin formation.

Various bioactive materials and macromolecules have been reported to decrease the extent of postoperative abdominal adhesions. Likewise, a number of methods for limiting the formation of surgical adhesion have been studied with some encouraging but often ambiguous results. However, most efforts made to avoid or reduce postoperative peritoneal adhesions have finally been abandoned. Among the methods used prevention of fibrin formation, reduction of fibrin formation, surface separation, and surgical techniques can be mentioned.

Numerous investigations have been carried out in which barriers are placed at a site of injury in order to prevent fibrin bridge formation between the injured tissue and neighboring organs. Such barriers include resorbable materials, such as enzymatically degradable oxidized re-

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generated cellulose, and slowly dissolving physiochemically crosslinked hydrogels of the Pluronic™ type.

Most methods of surface protection of exposed epithelial surfaces, whereby a postsurgical adhesion formation is limited, have also focused on providing wound separation by placing a material between the tissues. In addition, several types of viscous polymer solutions such as polyvinylpyrrolidone, sodium carboxymethyl cellulose, dextrans, and hyaluronic acid have been added before and/or at the end of surgery in order to control the wound healing events after the occurrence of the presumed tissue injuries. These solutions are supposed to act by increasing the lubrication and preventing the fibrin clots from adhering to other surfaces or by mechanically separating damaged tissues while they heal.

The employed polymeric solutions are mainly based on the viscosity of the high molecular weight polymer, which is intended to increase with increasing concentration. The polymer is often a polysaccharide as in US 4,994,277, in which a viscoelastic gel of biodegradable xanthan gum in a water solution for preventing adhesions between vital tissues is described. However, the major disadvantage of these polymers, when used for reducing for example peritoneal adhesions as protective coatings during surgery or surface separation agents after surgery, is that they do not significantly reduce adhesions because of their short residence time in the peritoneal cavity. The result is that subsequent surgeries have to be performed on the patient.

Less viscous polymer solutions have been used as a tissue protective coatings during surgery in order to maintain the natural lubricity of tissues and organs and to protect the enclosing membrane. Precoating for tissue protection and adhesion prevention includes coating tissues at the beginning of surgery before a significant tissue manipulation and irritation can occur and continuously through-



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out the operation so that a protective coating can be maintained on the tissues.

US 5,366,964 shows a surgical viscoelastic solution for promoting wound healing, which is used in direct contact with cells undergoing wound healing. The solution is intended for cell protection and cell coating during surgery and comprises one or several polymeric components. Hydroxypropylmethyl cellulose and chondroitin sulphate are supposed to lubricate the tissue, while sodium hyaluronate would provide viscoelastic properties to the solution.

Several agents of today for treating postsurgical adhesions contain hyaluronic acid. For example US 5,409,904 describes solutions which reduce cell loss and tissue damage intended for protecting endothelial cells during ophthalmic surgery. The compositions used are composed of a viscoelastic material comprising hyaluronic acid, chondroitin sulphate, modified collagen, and/or modified cellulose. In WO 9010031 a composition is described for preventing tissue adhesion after surgery containing dextran and hyaluronic acid act which substances are supposed to act synergistically. In WO 9707833 a barrier material for preventing surgical adhesions is shown, which comprises benzyl esters or covalently crosslinked derivatives of hyaluronic acid.

A hyaluronic acid based agent manufactured by Pharmacia under the trademark Healon and originally intended as an intraocular instillation has been found to be the most effective agent up to now. However, hyaluronic acid is isolated from cock's crests and is thus very expensive as well as potentially allergenic even in small quantities and even more for large surfaces such as the peritoneum which has an area of about two m<sup>2</sup>.

In WO 9903481 a composition for lubricating and separating tissues and biological membranes from adjacent membranes or adjacent cells or tissues is shown,

which comprises a hydrophobised polymer formed from a biologically acceptable water-soluble cationic polymer carrying covalently bound hydrophobic groups.

5 Likewise, water-insoluble biocompatible compositions are shown in EP 0,705,878, which comprise a polyanionic polysaccharide combined with a hydrophobic bio-absorbable polymer.

10 In US 6,235,313 a variety of polymers were compared for adhesive force to mucosa surfaces. Negatively charged hydrogels, such as alginate and carboxymethyl cellulose, with exposed carboxylic groups on the surface, were tested, as well as some positively-charged hydrogels, such as chitosan. The choice was based on the fact that most cell membranes are actually negatively charged. However, there  
15 is still no definite conclusion as to what the most important property is in order to obtain good bioadhesion to the wall of the gastrointestinal tract. For example, chitosan is considered to bind to a membrane by means of ionic interactions between positively charged amino groups  
20 on the polymer and negatively charged sialic acid groups on the membrane. Thus, polycationic molecules, such as chitosan and polylysine, have a strong tendency to bind to exposed epithelial surfaces since these generally have a negative net charge.

25 A main drawback of both these cationic molecules is that they exhibit toxic effects. For example, polylysine is considered to act as an inhibitor of the calcium channel by producing a conformational change, thereby inhibiting transmembrane ion fluxes.

30 The object of the invention is to provide a new approach of protecting exposed epithelial surfaces of a mammal, whereby the above-mentioned problems according to the state of the art are avoided or eliminated.

35 According to the invention, an effective amount of a water soluble cationic polymer, and a pharmaceutically

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acceptable carrier, is used in the manufacturing of a first drug in combination with an effective amount of a water soluble anionic polymer, and a pharmaceutically acceptable carrier, in the manufacturing of a second drug, which drugs  
5 are to be administered in sequence to a mammal for surface protection of exposed tissue surfaces.

The invention also refers to a kit comprising:

- (a) a first drug comprising an effective amount of a water soluble cationic polymer in a pharmaceutically  
10 acceptable carrier;
- (b) a second drug comprising an effective amount of a water soluble anionic polymer in a pharmaceutically acceptable carrier; and
- (c) means for administering the first and the second  
15 drug in sequence to a mammal for surface protection of exposed epithelial surfaces.

Without wishing to be bound to any theory, it is believed that the cationic polymer first binds to a membrane, and the anionic polymer subsequently binds to  
20 the cationic polymer, a complex between the cationic polymer and the anionic polymer being formed *in situ*.

It was surprisingly found that the toxic effects of the polycationic molecules could be eliminated by their fixation or immobilization in a "complex" with polyanionic  
25 macromolecules when these molecules were added immediately after the cationic molecules had bound to the exposed surface. Thus, the surface layer of bound cationic molecules are immobilized or "inactivated" by the subsequent administration of an anionic polymer. The cationic and  
30 anionic polymers can also be added to the exposed surface as a single entity, however with a reduced effect.

Furthermore, the anionic polymer and the cationic polymer in solution interact to significantly increase the viscosity of the polymeric material between for example  
35 membranes. This results in that the desired effect will



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remain and last for a longer time. An attachment of the material and an almost hard interface is obtained.

In fact, the material formed from the cationic and anionic polymers has a low solubility or is almost insoluble in dependence of the polymers used. The material formed effectively decreases experimental, postsurgical peritoneal adhesions by penetrating into the wound and isolating the same from the abdominal cavity. The material mechanically closes the wounds, rapidly covers other peritoneal surfaces and accumulates selectively around the injured site. Mesothelial cells can then grow over the wound during healing.

In addition, it was found that the material formed on the exposed epithelial surfaces had excellent biodegradability properties. After one month of administration the deposited material disappears and only in few cases can a minimal residual material be detected on intraabdominal postsurgical wounds.

In the drugs to be used according to the invention the water soluble ionic polymers are to be administered in pharmaceutically acceptable carrier. Such carriers are well known to those skilled in the art. Preferably, distilled water or buffered aqueous media are used, which contain pharmaceutically acceptable salts and buffers. Suitable salt solutions are PBS, PBSS, GBSS, EBSS, HBSS, and SBF.

The water soluble cationic polymer to be used according to the invention can be a natural polymer, such as a polysaccharide, a protein, or a polypeptide.

The polysaccharide can be chitosan or a cationic derivative of cellulose or starch.

A suitable protein, which can be used as a natural cationic polymer, is lysozyme.

However, it is preferred the water soluble cationic polymer is a polypeptide, especially a polyamino acid.

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Examples of cationic polyamino acids are polylysine, polyarginine, and polyhistidine.

The water soluble cationic polymer can also be a synthetic polymer.

- 5       The drug for administration of the water soluble cationic polymer should comprise 0.5-5 wt% of the same in the pharmaceutically acceptable carrier.

- 10       Likewise, the water soluble anionic polymer is a natural polymer that can be a polysaccharide, a protein, a polypeptide, or a polynucleotide. Suitable anionic polysaccharides are xanthan as well as alginic acid, hyaluronic acid, and polygalacturonic acid, or their salts. Anionic derivatives of cellulose and starch can also be used, such as carboxymethyl cellulose.

- 15       Insoluble cellulose and glucans can be derivatized by means of for example phosphorylation, sulfation, or amination to impart solubility to the natural polymer. Examples of such water soluble anionic polymers are dextran sulfate, cellulose sulfate, and sulfopropyl cellulose.

- 20       Alternatively, the anionic polymer can be N,O-carboxymethyl chitosan (NOCC), which has structural similarities with hyaluronic acid.

- 25       However, it is preferred the water soluble anionic polymer is a polypeptide, especially a polyamino acid. Examples of anionic polyamino acids are polyglutamate and polyaspartate.

- 30       The drug for administration of the water soluble anionic polymer should comprise 0.5-5 wt% of the same in the pharmaceutically acceptable carrier.

- It is preferred that the anionic polymer is administered after the cationic polymer has been administered. This, of course, also applies for the corresponding drugs. Preferably, the time span between the administrations should not exceed 10 min, most preferably less than 5 min.

The amount of anionic polymer should be administered in a stoichiometric excess relative the amount of cationic polymer. Preferably, the amount of anionic polyamino acid is 1.5-2 times the amount of cationic polyamino acid with reference to the number of anionic amino acid residues in relation to the number of cationic amino acid residues.

#### EXAMPLES

The invention will now be further described and illustrated by reference to the following examples. It should be noted, however, that these examples should not be construed as limiting the invention in any way.

##### Example 1. Adhesion prevention.

A reproducible and standardized rat and rabbit model was adopted. Forty eight female MRI mice weighing about 25-30 g were used to induce the adhesions and forty two for further tests. The animals were kept under standardized conditions and had free access to pellet and tap water.

Anesthesia was induced by ketamine 150 mg/kg (Ketalar, Parke Davis) and zylazine 7.5 mg/kg (Rompun, Bayer Sverige AB) intramuscular injection. After disinfection, a 25 mm long midline laparotomy was performed. Both peritoneal surfaces of the lateral abdominal wall were exposed, and 2x15 mm long sharp incisions were performed at the same distance from the midline, including the muscles. The wounds were immediately closed with 2x4 single sutures at equal distances by using 5.0 polypropylene (Prolene, Ethicon, Johnson & Johnson). The midline laparotomy was closed in two layers with a continuous 5.0 polypropylene suture. At the evaluation time an overdose of anesthetic was administered, the abdomen was totally opened through a U-shaped incision with its base to the right. The lengths of the adhesions were measured on both sides using a metal caliper, and data was expressed as percent wounds covered by adhesions.

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Aqueous solutions of 0.5% poly-L-glutamate, and poly-L-lysine were freshly made on the day of the experiment and stored in refrigerator until used. FITZ-labeled polylysine was mixed with polylysine in a proportion of 1:10 (wt). All chemicals and cell culture substrates were purchased from Sigma-Aldrich, St Louis, USA; fluorescent microparticles (Nile Blue Labeled) were bought from Microparticles GmbH., (Berlin, Germany).

The animals were divided randomly into 4 groups based on the treatment and the evaluation time. The control groups were intraperitoneally injected with 2 ml physiologic sodium chloride solution. Two treatment groups received 1 ml poly-L-lysine solution and 5 min later 1 ml poly-L-glutamate solution. One of the control and treatment groups (2x14 animals) was sacrificed one week after surgery and the lengths of the adhesions were calculated. The remaining two groups (2x10 animals) were kept for four weeks before they underwent the evaluation process.

The Kruskal Wallis test was used to determine the difference in adhesion amount among the different treated groups and the Mann Whitney U test was used to compare the individual groups.

A significant decrease in adhesion development was detected both one week and one month after the peritoneal challenge (\*\* $p \leq 0.001$ ) compared to the corresponding controls (Mann-Whitney U test). A marked (22%) though not significant ( $p = 0.235$ ) decrease was obtained after one month between the control groups, while there were no difference between the treated groups by that time.

No adhesions were found which were related to a heavy compound deposit in different locations from the wound itself. After 24 h, the animals that had been given both poly-L-lysine and poly-L-glutamate exhibited a massive protecting layer over the peritoneal wound, and thin film at the rest of peritoneal surface. However the FITZ-labeled



compound was only visible in the wound one day later and was detectable both over and inside the wound. The deposit was gradually rebuilt until the end of the 6 day observation period.

5

Example 2. Phagocytosis and particle ingestion index.

The time course of the phagocyte function was tested in vitro on peritoneal resident macrophages from mice after 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 16 h, 10 and 24 h incubation with poly-L-lysine + poly-L-glutamate (40 µg/ml) and/or fluorescent particles (1 µm).

Macrophage samples were taken by abdominal lavage with 10 ml ice cold DMEM-solution. The samples in medium were immediately centrifuged at 1200 rpm for 10 min. The 15 cells were resuspended in DMEM containing 10% FBS and penicillin/streptomycin and then plated on 48 wells cell culture plates;  $5 \times 10^5$  cells in each well. After 1.5 h non-adherent cells were washed away, particles (100/cell) together with test drugs (poly-L-lysine + poly-L-glutamate) 20 were added in a dose of 40 µg/ml to 12x5 wells, and particles only were added to the remaining 12x5 wells. Moreover negative controls were performed at each time point. The cells were incubated (37°C, 5% CO<sub>2</sub>) and detached and fixed at the evaluation time by using 250 µl 5 mM EDTA and 25 an equal volume of 2% paraformaldehyde. FACS analysis (FACScan, Becton Dickinson, San Jose, CA) was made, when cell size (forward scatter, FSC), granularity (side scatter, SSC) and fluorescence intensity (in FL3 channels) were recorded of  $1.5 \times 10^4$  cells in each measurement. In 30 manually defined gates the ratio phagocytosing cells/total macrophages was expressed in percent as mean of data from five wells at each time and treatment group (control and poly-L-lysine + poly-L-glutamate).

The non-treated cells incorporated more particles. 35 Thus, the maximum plateau (median) level of their fluorescence intensity (FL3) and SSC was set as 100%. All

measurements were expressed as the median percentage of the plateau level and termed particle ingestion index, since it refers to the amount of particles ingested.

5 The Mann Whitney U test was used to check the plateau of phagocytosis and the Wilcoxon Signed Ranks test was used to test the difference in the phagocytosis and particle ingestion index between the treatment pairs (control and poly-L-lysine + poly-L-glutamate, respectively).

10 While the phagocytosis index of the non-treated macrophages reached the plateau of phagocytosis about 5 h (the difference between 4 and 5 h decreased below the insignificant level,  $p=1$ ), the treated population required 8 h for the same effect. (The difference between 8 and 10 h was insignificant,  $p=0.058$ ). A low but significant  
15 ( $p=0.043$ ) difference was obtained in the phagocytosis index after 24 h (97.3% and 94.3%, respectively).

The time course for the ingestion index, which refers to the number of particles phagocytosed by macrophages became significant between 1 and 2 h ( $p=0.008$ ). The control  
20 cell population reached the plateau between 16 and 24 h (insignificant difference between the index at 12 and 24 h ( $p=0.841$ ) while the treated cell population did not reach the plateau at all during the first 24 h studied. Furthermore, the number of ingested particles were significantly  
25 lower in the treated group at all times ( $p=0.043$ ).

Flow cytometry verified that macrophages phagocyte the test compound particles, which resulted in significant cell growth and large phagocytic vacuoles.

### 30 Example 3. Transmission electron microscopy.

Peritoneal macrophages were harvested from two healthy non-treated animals as described above and plated on cell culture plates (Thermanox, Naperville, IL, USA). The cells were washed away after 1.5 h and poly-L-lysine +  
35 poly-L-glutamate (40 µg/ml) in supplemented DMEM solution were added in sequence followed by a 24 h incubation. The

incubation medium was removed and the cells were fixed in 2.5% phosphate buffered glutaraldehyde was followed by rinsing in Millonig's phosphate solution. Samples were postfixed in 1% osmium tetroxide and subsequently de-  
5 hydrated with graded series of ethanol, which was followed by embedding in Araldite 502 kit. Vertical sections were obtained with a diamond knife and stained with uranyl acetate and lead citrate in a LKB Ultrastainer. Samples were examined in a JEOL 1200 EX transmission electron  
10 microscope (TEM).

Electron microscopy verified that macrophages phagocyte the test compound particles, resulted in significant cell growth, and large phagocytic vacuoles.

15 Example 4. Scanning electron microscopy.

Peritoneal swabs and wounds were taken from eight treated (4) and non-treated (4) animals after one and seven days of surgery and cell cultures were conducted as above. The samples were fixed in 2.5% phosphate buffered glutar-  
20 aldehyde at room temperature and then post-fixed in 1% OsO<sub>4</sub>. The samples were dehydrated in acetone, critical point dried and sputter-coated with gold before being studied in a LEO 420 electron microscope.

SEM data showed that mesothelial cells covered the  
25 compound surface from the first day.

Example 5. Histology.

Eight animals were opened and then injected intra-peritoneally with poly-L-lysine + poly-L-glutamate. At the  
30 postoperative first, second, third, and sixth days, two animals were sacrificed and the wounds were excised. They were rapidly frozen and embedded, and the block obtained was immediately cut into slices of 7 µm. The slices were allowed to dry in dark for 30 min at room temperature and  
35 were then stained with 100 µg/l 4'6'-diamino-2-phenylindole hydrochloride (DAPI) solution for 10 min. Fluorescent

microscopy was performed with both a FITZ and a DAPI filter, and images were digitally merged (OpenLab, Improvision). Macro photo was made about the excised wounds by using trans-illumination, mixed ambient room light, and UV illumination.

The histological studies showed that the added material was present in the wound from the first day. Furthermore, more and more cells were detected for each day until the matrix was completely rebuilt.

#### Example 6. Biodegradation.

Healthy nonoperated animals were treated intraperitoneally as in Example 1 and sacrificed after two months.

No visible remains of poly-L-lysine and poly-L-glutamate could be detected. The biodegradability is supported by findings that at one month's follow up the same results were obtained by using a double dose of poly-L-lysine + poly-L-glutamate, although that caused some additional adhesions related to the compound at evaluation on the 7<sup>th</sup> day.

#### Comparative example 1. Biodegradation.

Aqueous solutions of 1% and 2% lysozyme, poly-L-glutamate, poly-L-lysine, and poly-L-glutamate, and 0.25% of hyaluronic acid were freshly made. Solutions of lysozyme, polyglutamate, lysozyme + polyglutamate and polylysine + polyglutamate were then administered to animals as in Example 1.

The extent of abdominal adhesions one week after surgery significantly decreased in the four treated groups ( $p \leq 0.001$ ) as compared to controls. However, no significant change in response was obtained with hyaluronic acid ( $p = 0.264$ ). The combinations poly-L-lysine/lysozyme seemed to result in an insoluble product.



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Comparative example 2. Effect of poly-L-lysine alone.

An aqueous solution of 0.5% poly-L-lysine was freshly made and administered to animals as in Example 1.

- 5 Such an administration of poly-L-lysine alone resulted in convulsions and death within 30 min, i.e. before they woke up from the anesthesia. The symptoms seemed to be related to the effect of opening calcium channels, plasma  $\text{Ca}^{++}$  levels being rapidly decreased.

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## CLAIMS

1. Use of an effective amount of a water soluble cationic polymer, and a pharmaceutically acceptable carrier, in the manufacturing of a first drug and an effective amount of a water soluble anionic polymer, and a pharmaceutically acceptable carrier, in the manufacturing of a second drug, wherein said first and said second drug are to be administered in sequence to a mammal for surface protection of exposed biological tissue surfaces.
2. Use as in claim 1, characterized in that said water soluble cationic polymer is a natural polymer.
3. Use as in claim 2, characterized in that said natural cationic polymer is a polysaccharide, a protein, or a polypeptide.
4. Use as in claim 3, characterized in that said polysaccharide is chitosan or a cationic derivative of cellulose or starch.
5. Use as in claim 3, characterized in that said protein is lysozyme.
6. Use as in claim 3, characterized in that said polypeptide is a cationic polyamino acid.
7. Use as in claim 6, characterized in that said cationic polyamino acid is polylysine, polyarginine, or polyhistidine.
8. Use as in claim 1, characterized in that said water soluble cationic polymer is a synthetic polymer.
9. Use as in claim 1, characterized in that said first drug comprises 0.5-5 wt% of said water soluble cationic polymer in said pharmaceutically acceptable carrier.
10. Use as in claim 1, characterized in that said water soluble anionic polymer is a natural polymer.

11. Use as in claim 10, characterized in that said natural anionic polymer is a polysaccharide, a protein, a polypeptide, or a polynucleotide.

5 12. Use as in claim 11, characterized in that said polysaccharide is xanthan, alginic acid, hyaluronic acid, or polygalacturonic acid, or a salt thereof, a carragenan, or an anionic derivative of cellulose or starch.

10 13. Use as in claim 12, characterized in that said anionic cellulose derivative is carboxymethyl cellulose.

14. Use as in claim 11, characterized in that said polypeptide is an anionic polyamino acid.

15 15. Use as in claim 14, characterized in that said anionic polyamino acid is polyglutamate or polyaspartate.

20 16. Use as in claim 1, characterized in that said second drug comprises 0.5-5 wt% of said water soluble anionic polymer in said pharmaceutically acceptable carrier.

25 17. Use as in claim 6 and 14, characterized in that the amount of said anionic polyamino acid in said second drug exceeds the amount of said cationic polyamino acid in said first drug with reference to the number of anionic amino acid residues in relation to the number of cationic amino acid residues.

30 18. Use as in claim 1, characterized in that said pharmaceutically acceptable carrier is distilled water or a salt solution.

19. Use as in claim 1, characterized in that said second drug is to be administered after said first drug is to be administered.

35 20. Use as in claim 19, characterized in that said second drug is to be administered less than 5 min after said first drug is to be administered.

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## 21. A kit comprising:

(d) a first drug comprising an effective amount of a water soluble cationic polymer and a pharmaceutically acceptable carrier;

5 (e) a second drug comprising an effective amount of a water soluble anionic polymer and a pharmaceutically acceptable carrier; and

10 (f) means for administering said first and said second drug in sequence to a mammal for surface protection of exposed epithelial surfaces.



## ABSTRACT

The invention refers to the use of an effective amount of a water soluble cationic polymer, and a pharmaceutically acceptable carrier, in the manufacturing of a first drug and an effective amount of a water soluble anionic polymer, and a pharmaceutically acceptable carrier, in the manufacturing of a second drug. The drugs are to be administered in sequence to a mammal for surface protection of exposed biological tissue surfaces.

The invention also refers to a kit comprising a first drug comprising an effective amount of a water soluble cationic polymer and a pharmaceutically acceptable carrier; a second drug comprising an effective amount of a water soluble anionic polymer and a pharmaceutically acceptable carrier; and means for administering the drugs in sequence to a mammal.